REPORT

The BlyS/BAFF family of ligands and receptors: key targets in the therapy and understanding of autoimmunity

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The B lymphocyte stimulator (BlyS; also termed BAFF) family of ligands and receptors plays a central role in B lymphocyte development, selection, and homeostasis. Members of this family can independently influence different B cell subsets, because the interactions between the two ligands and three receptors vary, and the receptors themselves are differentially expressed among developing, naive, and antigen experienced B cell subsets. These properties prompt careful assessment of how ablative therapies may influence the behaviour of upstream or downstream B lineage populations, as well as how the implementation and expectations of therapeutics targeting BlyS family members must be guided by knowledge of the B cell subsets contributing to pathogenesis.

In the relatively short period since their initial description,^{1,2} the B lymphocyte stimulator (BlyS; also termed BAFF) family of ligands and receptors has assumed a prominent position in fundamental B lymphocyte biology, leading to considerable review and commentary.^{3–8} A growing body of evidence suggests these molecules play essential roles in the selection, differentiation, and homeostatic control of nearly all peripheral B cell populations. In addition, BlyS and its receptors have been implicated in humoral autoimmunity, largely based on clinical studies linking elevated BlyS levels with humoral autoimmune syndromes (reviewed in references 9–13), as well as on studies in transgenic mouse model systems. Accordingly, how these molecules and their activities may impact targeted therapeutics aimed at B cell subsets, as well as how they and their downstream signalling pathways might serve as targets per se, warrants careful scrutiny.

THE BlyS FAMILY OF LIGANDS AND RECEPTORS

The BlyS family consists of two ligands, a proliferation inducing ligand (APRIL) and BlyS; and three receptors, transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), B cell maturation antigen (BCMA), and BlyS receptor 3 (BR3; also termed BAFFR). Similar to other tumour necrosis factor (TNF) ligands, both BlyS and APRIL form active, soluble trimers after furin cleavage. The three receptors also share features with other TNF superfamily members, although BR3 is unusual; having only one cysteine-rich domain, as well as unique cysteine residue spacing and hydrophobicity in the ligand binding region—presumably explaining its selective interaction with BlyS.^{14–16}

Appreciation of two general features of the receptors and ligands comprising this family is crucial to understanding how it can mediate such diverse and independent activities. Firstly, the interactions between ligands and receptors vary: BlyS can interact with all three receptors, although most

strongly with BR3, whereas APRIL can interact strongly with TACI or BCMA, but not at all with BR3. Secondly, the receptors themselves are differentially expressed across various functional B cell subsets. For example, BR3 and TACI are expressed among transitional, follicular, and marginal zone B cells, whereas among long lived marrow plasma cells, these two receptors are downregulated and BCMA is expressed instead.

Thus, the permutations afforded by varied expression patterns and ratios of the three receptors, combined with the differential binding characteristics of the two ligands, affords the potential to independently influence different B cell subsets. From the standpoint of targeted therapies, this raises two overarching and equally important questions:

- How might the elimination of large cross-sections of these populations influence the behaviour of upstream or downstream populations either during or after therapy?
- What populations are most likely to be influenced by therapeutics targeting particular ligands or receptors of this family, and how should this temper expectation?

BlyS–BR3 INTERACTIONS CONTROL TRANSITIONAL SUCCESS AND MATURE PRIMARY B CELL SURVIVAL

The most detailed characterisations of BlyS activities have been among developing and primary B cells. Current models of B cell differentiation hold that following B cell receptor (BCR) expression at the late bone marrow pre-B stage, cells enter the immature bone marrow pool and are subsequently exported to the periphery as transitional B cells, where they undergo final maturation prior to entering the follicular or marginal zone pools.^{17–22} BlyS binding and responsiveness begins concomitant with BCR expression, reflecting the upregulation of BR3 and TACI during the immature bone marrow B cell stage.²³ Extensive evidence from mutant, knockout, and transgenic mouse models indicates that the likelihood recent marrow émigrés will survive transitional differentiation depends on BlyS signalling via BR3. Thus, in functionally inactive mutants and BR3 knockouts, B cell production is severely truncated at the late transitional stages, whereas neither TACI nor BCMA knockouts display reductions in primary B cell numbers.^{24–27} Mature primary cells in the follicular and marginal zone subsets also display a profound dependence on BlyS for their survival and compete for available BlyS to survive, as demonstrated by kinetic studies in mixed marrow chimeras and BR3 haploinsufficient mice.^{23, 26}

Abbreviations: APRIL, a proliferation inducing ligand; BCMA, B cell maturation antigen; BCR, B cell receptor; BlyS, B lymphocyte stimulator; BR3, BlyS receptor 3; TACI, transmembrane activator and calcium modulator and cyclophilin ligand interactor; TNF, tumour necrosis factor

AVAILABLE BlyS AFFORDS HOMOEOSTATIC ADJUSTMENT OF TRANSITIONAL CELL THROUGHPUT, ADJUSTING THE STRINGENCY OF TRANSITIONAL CELL NEGATIVE SELECTION

Maturation at the marrow periphery interface involves stringent selective processes. Under normal circumstances, only about 10% of the immature B cells generated survive to exit the bone marrow as transitional cells; and of these, only about half ultimately survive to join the follicular or marginal zone pools.^{18 19 21} A substantial proportion of these cell losses reflects negative selection, presumably driven by the interactions of newly expressed BCRs with self-ligands. Evidence from transgenic models suggest that high avidity self-reactive clonotypes tend to be lost at the bone marrow immature stages, whereas emerging cells engaged in low avidity self-interactions, including so-called “polyreactive” clonotypes, are eliminated at the transitional checkpoint.^{28–33} Recent studies have linked these negative selective processes with peripheral homoeostasis, by showing that the stringency of transitional negative selection can be relaxed in the presence of excess BlyS.^{34 35} In these studies, clonotypes normally lost at the transitional checkpoint were afforded entrance to the mature peripheral pools under conditions of excess BlyS, and the likelihood that BlyS could “rescue” these clonotypes was inversely related to the avidity for self-antigen. These findings have been recently extended to anti-dsDNA specificities in the 3H9 transgenic model by Hondowicz *et al* (manuscript submitted), with similar results. Importantly, negative selection at the immature bone marrow stage is not subject to adjustment by available BlyS levels,³⁵ consistent with the very low BlyS binding activity at these stages.

Together, these considerations have led us to propose a model,³⁶ whereby selection is divided into two phases. The first occurs in the bone marrow and relies on BCR strength alone to determine whether a clone faces elimination versus further differentiation and exit. The second occurs in the peripheral transitional cell pools, where throughput is tied not only to BCR signal strength but to the maintenance of adequate mature, primary B cell numbers, with available BlyS serving as an indicator of unfilled biological space.

APPRECIATION OF HOW TRANSITIONAL CELL SELECTION AND THROUGHPUT ARE BALANCED IMPACTS ON THINKING ABOUT ABLATIVE THERAPIES

The ability to adjust the threshold for elimination during transitional cell differentiation links selection and homoeostasis, and has substantial implications for scenarios in which the primary pool's size is reduced precipitously. In these circumstances, the systemic concentration of available BlyS will increase, likely reducing selective stringency. Accordingly, it is worth asking whether strategies targeting large peripheral B cell populations can engender relaxed transitional cell selection during the window of B lymphoid auto-reconstitution following cessation of therapy. Whether this is indeed an issue might be addressed by tracking the proportional representation of known clonotypes in the transitional versus re-emerging follicular populations. If clonotypes normally eliminated at the transitional stage are observed in the follicular and marginal zone compartments during early reconstitution, then an increased risk of resurgent autoreactivity may result. In this regard, it is important to note that the re-emergence of frank autoimmunity from such a mechanism will be probabilistic, since longevity and maturation of these cells per se will not necessarily result in autoantibody formation or autoantigen presentation. Nonetheless, increasing the probability of such events in predisposed individuals may be particularly worrisome. Should this prove the case, antagonists or neutralising

biologicals that titre available BlyS during the initial phases of lymphoid auto-reconstitution may restore normal selective thresholds.

EXOGENOUS ACTIVATION CUES ENGENDER SHIFTS IN BlyS FAMILY RECEPTOR EXPRESSION IN VITRO AND IN VIVO

An understanding of how the BlyS family members influence B cells after activation and further differentiation are only now beginning to emerge. Since activated and memory B cell subsets comprise independently regulated homoeostatic and selective niches, the question arises as to how these cells are freed from competition with the naive quiescent pools, and whether BlyS family members continue to play a role in their independent homoeostatic control. Recent findings from our laboratory and others suggest that activation cues through innate and adaptive receptors modulate the levels and array of BlyS receptors on activated cells. In general, activation cues that engender differentiation to end-stage antibody forming cells appear to favour the upregulation of TACI, whereas those that engender germinal centre participation preferentially increase BR3. Finally, long lived plasma memory cells, presumably the endproduct of the germinal centre reaction, appear to have lost TACI and BR3 expression and instead express BCMA.³⁷ These observations suggest a scenario in which independent homoeostatic niches are characterised by BlyS receptor expression signatures that determine the nature and outcome of ligand binding, as well as the competing cohort.⁶ For example, BlyS–BR3 interactions will determine naive B cell numbers, whereas APRIL–BCMA interactions are likely the key homoeostatic axis for memory plasma cell populations.

HOW DOES TRIAGE OF ACTIVATED CELLS INTO ALTERNATIVE HOMOEOSTATIC NICHES COLOUR THOUGHT ABOUT THE IMPACT TARGETS OF ABLATIVE THERAPIES?

Although the details of how BlyS receptor signatures delineate and influence antigen experienced B cell populations remain unclear, these initial observations have implications for current and future targeted biological therapeutics. On the one hand, knowledge of these differing patterns may afford refined ability to spare inoffensive populations, based on which homoeostatic axis is targeted. For example, if pathogenic autoantibody emanates from the memory plasma cell pool, then therapies designed to specifically target either BCMA per se or ligands that favour BCMA, might prove effective, without substantial impact on primary follicular and marginal zone pools. On the other hand, these observations also indicate that biological targets must be carefully assessed in terms of the subset most likely influenced, since offending populations may escape therapeutics targeting only niches governed by BlyS–BR3 interactions. Accordingly, careful assessments of the B cell populations contributing directly to disease should allow the informed design of therapeutics based on targeting homoeostatic niches defined by the BlyS family of ligands and receptors.

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REFERENCES

- 1 Schneider P, MacKay F, Steiner V, Hofmann K, Bodmer JL, Holler N, *et al*. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J Exp Med* 1999;**189**:1747–56.

- 2 **Moore PA**, Belvedere O, Orr A, Pieri K, LaFleur DW, Feng P, *et al*. BlyS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science* 1999;**285**:260–3.
- 3 **Mackay F**, Browning JL. BAFF: a fundamental survival factor for B cells. *Nat Rev Immunol* 2002;**2**:465–75.
- 4 **Melchers F**. Actions of BAFF in B cell maturation and its effects on the development of autoimmune disease. *Ann Rheum Dis* 2003;**62**(suppl 2):ii25–7.
- 5 **Cancro MP**. The BlyS family of ligands and receptors: an archetype for niche-specific homeostatic regulation. *Immunol Rev* 2004;**202**:237–49.
- 6 **Crowley JE**, Trembl LS, Stadanlick JE, Carpenter E, Cancro MP. Homeostatic niche specification among naive and activated B cells: a growing role for the BlyS family of receptors and ligands. *Semin Immunol* 2005;**17**:193–9.
- 7 **Kalied SL**. The role of BAFF in immune function and implications for autoimmunity. *Immunol Rev* 2005;**204**:43–54.
- 8 **Schneider P**. The role of APRIL and BAFF in lymphocyte activation. *Curr Opin Immunol* 2005;**17**:282–9.
- 9 **Nardelli B**, Moore PA, Li Y, Hilbert DS. B lymphocyte stimulator (BlyS): a therapeutic trichotomy for the treatment of B lymphocyte diseases. *Leuk Lymphoma* 2002;**43**:1367–73.
- 10 **Zhou T**, Zhang J, Carter R, Kimberley R. BlyS and B cell autoimmunity. *Curr Dir Autoimmun* 2003;**6**:21–37.
- 11 **Stahl W**. A therapeutic role for BlyS antagonists. *Lupus* 2004;**13**:317–22.
- 12 **Jelinek DF**, Darce JR. Human B lymphocyte malignancies: exploitation of BlyS and APRIL and their receptors. *Curr Dir Autoimmun* 2005;**8**:266–88.
- 13 **Mackay F**, Sierro F, Grey ST, Gordon TP. The BAFF/APRIL system: an important player in systemic rheumatic diseases. *Curr Dir Autoimmun* 2005;**8**:243–65.
- 14 **Kayagaki N**, Yan M, Seshasayee D, Wang H, Lee W, French DM, *et al*. BAFF/BlyS receptor 3 binds the B cell survival factor BAFF ligand through a discrete surface loop and promotes processing of NF-kappaB2. *Immunity* 2002;**17**:515–24.
- 15 **Hymowitz SG**, Patel DR, Wallweber HJ, Runyon S, Yan M, Yin J, *et al*. Structures of APRIL-receptor complexes: like BCMA, TACI employs only a single cysteine-rich domain for high affinity ligand binding. *J Biol Chem* 2005;**280**:7218–27.
- 16 **Gordon NC**, Pan B, Hymowitz SG, Yin J, Kelley RF, Cochran AG, *et al*. BAFF/BlyS receptor 3 comprises a minimal TNF receptor-like module that encodes a highly focused ligand-binding site. *Biochemistry* 2003;**42**:5977–83.
- 17 **Allman DM**, Ferguson SE, Cancro MP. Peripheral B cell maturation. I. Immature peripheral B cells in adults are heat-stable antigen(hi) and exhibit unique signaling characteristics. *J Immunol* 1992;**149**:2533–40.
- 18 **Allman DM**, Ferguson SE, Lentz VM, Cancro MP. Peripheral B cell maturation. II. Heat-stable antigen(hi) splenic B cells are an immature developmental intermediate in the production of long-lived marrow-derived B cells. *J Immunol* 1993;**151**:4431–44.
- 19 **Rolink AG**, Andersson J, Melchers F. Characterization of immature B cells by a novel monoclonal antibody, by turnover and by mitogen reactivity. *Eur J Immunol* 1998;**28**:3738–48.
- 20 **Loder F**, Mutschler B, Ray RJ, Paige CJ, Sideras P, Torres R, *et al*. B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals. *J Exp Med* 1999;**190**:75–89.
- 21 **Allman D**, Lindsley RC, DeMuth W, Rudd K, Shinton SA, Hardy RR. Resolution of three nonproliferative immature splenic B cell subsets reveals multiple selection points during peripheral B cell maturation. *J Immunol* 2001;**167**:6834–40.
- 22 **Cancro MP**. Peripheral B-cell maturation: the intersection of selection and homeostasis. *Immunol Rev* 2004;**197**:89–101.
- 23 **Hsu BL**, Harless SM, Lindsley RC, Hilbert DM, Cancro MP. Cutting edge: BlyS enables survival of transitional and mature B cells through distinct mediators. *J Immunol* 2002;**168**:5993–6.
- 24 **Gross JA**, Dillon SR, Mudri S, Johnston J, Littau A, Roque R, *et al*. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease. impaired B cell maturation in mice lacking BlyS. *Immunity* 2001;**15**:289–302.
- 25 **Schiemann B**, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, *et al*. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science* 2001;**293**:2111–14.
- 26 **Harless SM**, Lentz VM, Sah AP, Hsu BL, Clise-Dwyer K, Hilbert DM, *et al*. Competition for BlyS-mediated signaling through Bcma/BR3 regulates peripheral B lymphocyte numbers. *Curr Biol* 2001;**11**:1986–9.
- 27 **Yan M**, Brady JR, Chan B, Lee WP, Hsu B, Harless S, *et al*. Identification of a novel receptor for B lymphocyte stimulator that is mutated in a mouse strain with severe B cell deficiency. *Curr Biol* 2001;**11**:1547–52.
- 28 **Goodnow CC**, Crosbie J, Jorgensen H, Brink RA, Basten A. Induction of self-tolerance in mature peripheral B lymphocytes. *Nature* 1989;**342**:385–91.
- 29 **Hartley SB**, Crosbie J, Brink R, Kantor AB, Basten A, Goodnow CC. Elimination from peripheral lymphoid tissues of self-reactive B lymphocytes recognizing membrane-bound antigens. *Nature* 1991;**353**:765–9.
- 30 **Cyster JG**, Hartley SB, Goodnow CC. Competition for follicular niches excludes self-reactive cells from the recirculating B-cell repertoire. *Nature* 1994;**371**:389–95.
- 31 **Fulcher DA**, Basten A. Reduced life span of anergic self-reactive B cells in a double-transgenic model. *J Exp Med* 1994;**179**:125–34.
- 32 **Fulcher DA**, Lyons AB, Korn SL, Cook MC, Koleda C, Parish C, *et al*. The fate of self-reactive B cells depends primarily on the degree of antigen receptor engagement and availability of T cell help. *J Exp Med* 1996;**183**:2313–28.
- 33 **Cyster JG**. Signaling thresholds and interclonal competition in preimmune B-cell selection. *Immunol Rev* 1997;**156**:87–101.
- 34 **Lesley R**, Xu Y, Kalied SL, Hess DM, Schwab SR, Shu HB, *et al*. Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. *Immunity* 2004;**20**:441–53.
- 35 **Thien M**, Phan TG, Gardam S, Amesbury M, Basten A, Mackay F, *et al*. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. *Immunity* 2004;**20**:785–98.
- 36 **Miller JP**, Stadanlick JE, Cancro MP. Space, selection and surveillance: setting boundaries with BlyS. *J Immunol* 2006;**176**:6405–10.
- 37 **O'Connor BP**, Raman VS, Erickson LD, Cook WJ, Weaver LK, Ahonen C, *et al*. BCMA is essential for the survival of long-lived bone marrow plasma cells. *J Exp Med* 2004;**199**:91–8.